iGEM TU/e 2014
Biomedical Engineering

Eindhoven University of Technology
Room: Ceres 0.04
Den Dolech 2, 5612 AZ Eindhoven
The Netherlands
Tel. no. +31 50 247 55 59
2014.igem.org/Team:TU_Eindhoven

Cell viability assay

This protocol is designed to check the viability of the bacteria after a click reaction with a specific DBCO functionalized molecule.

Technische Universiteit
Eindhoven
University of Technology

Table of contents

Title
Cell viability assay

1 Culturing & Protein Expression

- Culture the bacteria and induce protein expression according to the protocol Protein Expression.

2 Prepare stock solutions

- 5 mM DBCO-TAMRA
- 5 mM PEG 3350
- Buffer: PBS

3 Preparing reaction samples

- Prepare following tubes:

Tube name	[DBCO & PEG no DBCO]	Cells [10^9]	DBCO volume to add (µL) (5 mM)	PEG (no DBCO)
DBCO 1	30 µM	200 µL	1.21	0
DBCO 2	30 µM	200 µL	1.21	0
PEG 1	30 µM	200 µL	0	1.21
PEG 2	30 µM	200 µL	0	1.21

- React the tubes for 2h at 500 RPM in shaking block at 4°C. Make sure you cover it with aluminum foil.

4 Preparing agar plates

- Take 240 mL of autoclaved agar and add 240 µL chloramphenicol (25 µg/mL) and 240 µL kanamycine (30 µg/mL).
- Pour 12 agar plates.
- Put the agar plates into the 37 °C incubator 15 minutes before plating the cells.

5 Diluting the samples

- Dilute the reacted samples according to the following scheme.

Tube	PBS	Sample name:	Volume sample:	Concentration:
Dilution DBCO 1_1	999 µL	DBCO 1	1 µL	10^6 cells / mL
Dilution DBCO 1_2	495 µL	Dilution DBCO 1_1	5 µL	10^4 cells / mL
Dilution DBCO 1_3	180 µL	Dilution DBCO 1_2	20 µL	10^3 cells / mL
Dilution DBCO 1_4	180 µL	Dilution DBCO 1_3	20 µL	10^2 cells / mL
Dilution DBCO 2_1	999 µL	DBCO 2	1 µL	10^6 cells / mL
Dilution DBCO 2_2	495 µL	Dilution DBCO 2_1	5µL	10^4 cells / mL
Dilution DBCO 2_3	180 µL	Dilution DBCO 2_2	20 µL	10^3 cells / mL
Dilution DBCO 2_4	180 µL	Dilution DBCO 2_3	20 µL	10^2 cells / mL
Dilution PEG 1_1	999 µL	PEG 1	1 µL	10^6 cells / mL
Dilution PEG 1_2	495 µL	Dilution PEG 1_1	5 µL	10^4 cells / mL
Dilution PEG 1_3	180 µL	Dilution PEG 1_2	20 µL	10^3 cells / mL
Dilution PEG 1_4	180 µL	Dilution PEG 1_3	20 µL	10^2 cells / mL
Dilution PEG 2_1	999 µL	PEG 2	1 µL	10^6 cells / mL
Dilution PEG 2_2	495 µL	Dilution PEG 1_1	5 µL	10^4 cells / mL
Dilution PEG 2_3	180 µL	Dilution PEG 1_2	20 µL	10^3 cells / mL
Dilution PEG 2_4	180 µL	Dilution PEG 1_3	20 µL	10^2 cells / mL

6 Plating the bacteria

- Plate the following samples on the prepared agar plates.

Tube	Volume for plating	Number of bacteria
Dilution DBCO 1_2	100 µL	1000
Dilution DBCO 1_3	100 µL	100
Dilution DBCO 1_4	100 µL	10
Dilution DBCO 2_2	100 µL	1000
Dilution DBCO 2_3	100 µL	100
Dilution DBCO 2_4	100 µL	10
Dilution PEG 1_2	100 µL	1000
Dilution PEG 1_3	100 µL	100
Dilution PEG 1_4	100 µL	10
Dilution PEG 2_2	100 µL	1000
Dilution PEG 2_3	100 µL	100
Dilution PEG 2_4	100 µL	10

- Incubate the plates for ~16 hours on 37 °C.

7 Analyzing

- The following day take the plates out of the incubator.
- Count the number of colonies and compare them with the original amount of bacteria plated.